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Technical methods

An improved container for cells preserved by freezing

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The use of storage at low temperature to preserve cells for long periods has already proved valuable in medicine and biology and further applications of this technique may be expected. Though both fast and slow cooling have their advocates (see Smith, 1954, for a full comparative review), the most commonly used method employs glycerol (Polge, Smith, and Parkes, 1949) or dimethyl sulphoxide (Lovelock and Bishop, 1959; Ashwood-Smith, 1961) as a protective agent; cooling is carried out according to the biphasic technique of Polge and Lovelock (1952). The frozen material is stored at −79°C. with solid CO_2 and thawed rapidly in a water-bath. This paper describes a container of new design for use in this process.

The requirements to be met are as follows:

HEAT TRANSFER Rapid heat transfer is desirable. During cooling this helps to produce a precisely controlled curve and it also permits the fast rate of thawing which is generally considered vital (Smith, 1954; Meryman, 1960).

PENETRATION BY CARBON DIOXIDE It is important to exclude carbon dioxide from the stored material (Smith, personal communication).

CONVENIENCE The ideal container should be simple to seal, label and open, and easy to sterilize.

ECONOMY It should be cheap and occupy the minimum storage space.

Since thawing is probably the most critical phase of the process, thawing curves were recorded with direct-writing thermocouples sealed into a number of different containers (Fig. 1). It is clear that the essential factor in heat-exchange is the surface area: volume ratio; when this falls below 4 sq. cm./ml. the thawing time increases sharply. For volumes exceeding 10 ml. only a flat sachet provides acceptable thawing times.

Permeability to carbon dioxide was studied by filling the containers with a carbonate/bicarbonate buffer and measuring their carbon dioxide content at various intervals (Fig. 2) by the method of Skeggs (1960). Glass ampoules and sachets incorporating aluminium foil

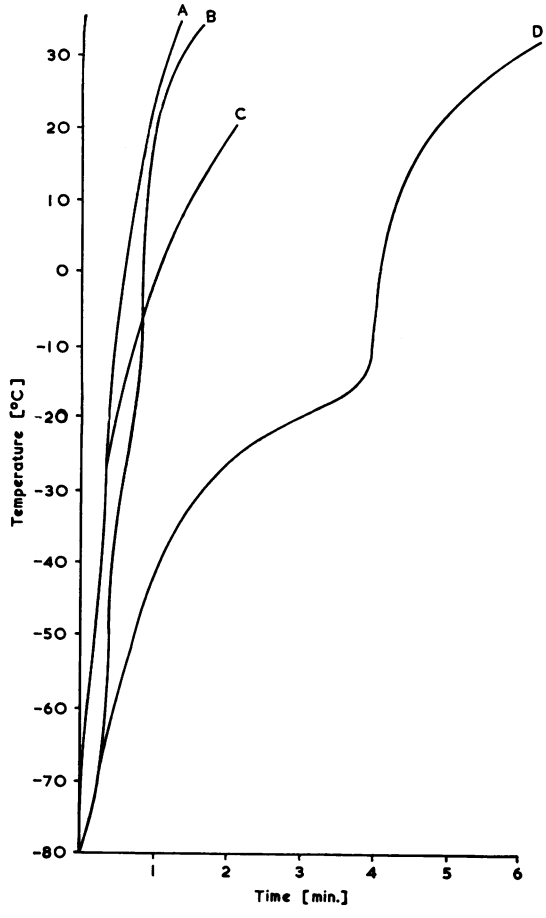

FIG. 1. *Thawing curves.*

The containers were:

A *An aluminium/polyethylene sachet containing 25 ml. of human blood with 15% glycerol. Surface area: volume ratio (SA : V) = 7·2 sq. cm./ml.*

B *A 10 ml. glass ampoule containing glycerolized blood, SA : V = 4·4 sq. cm./ml.*

C *A Fenwal bag containing 100 ml. of glycerolized blood, SA : V = 3·8 sq. cm./ml.*

D *A polyethylene ampoule containing 25 ml. of glycerolized blood, SA : V = 2·3 sq. cm./ml.*

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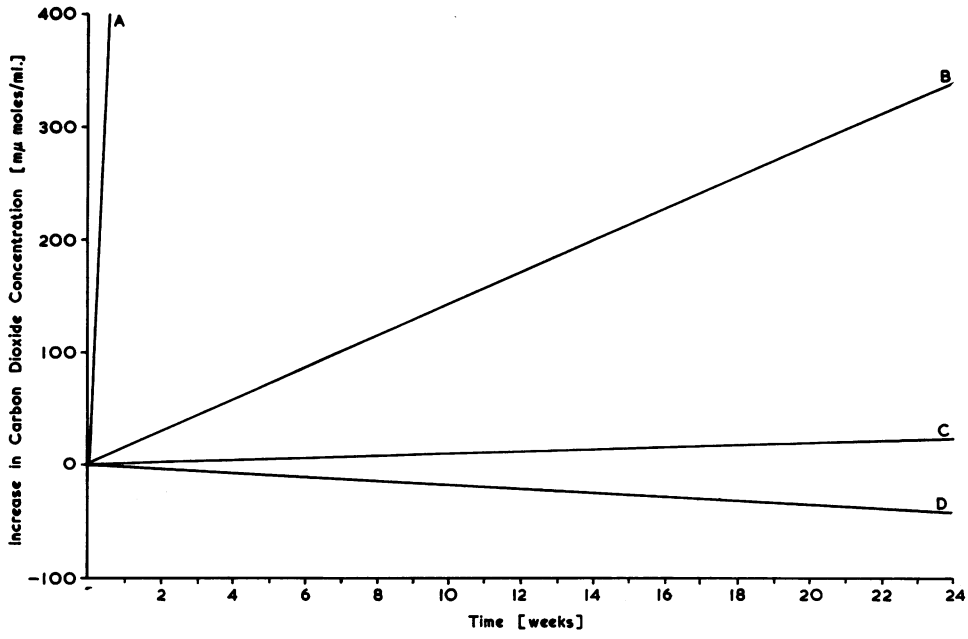

FIG. 2. *Carbon dioxide penetration. The lines were fitted by the method of least squares. The containers tested were:*

A *Fenwal bags containing 100 ml.* B *Polyethylene ampoules containing 25 ml.*
C *Aluminium/polyethylene sachets containing 10 ml.* D *Glass ampoules containing 10 ml.*

Lines C *and* D *do not deviate significantly from the horizontal.*

showed no detectable increase during 24 weeks; identical sachets made of polyethylene only (up to 0·25 mm. thick) accumulated carbon dioxide too rapidly to appear on the graph. Fenwal bags were very little better. Polyethylene ampoules, with their much thicker walls, allowed slow penetration of carbon dioxide.

Both of these tests show the superiority of a sachet made from a laminate of aluminium and polyethylene. The material tested consists of 18 lb. paper/50 g. polyethylene/0·001 in. aluminium foil/200 g. polyethylene. The sachets are readily sealed by heat[1], the paper surface is ideal for labelling, and they can be opened with scissors. They were sterilized by *x*-irradiation. The price is low, since sachets can be made in the laboratory from a roll of the material. They are very economical in storage space, but the cooling process may be complicated by the awkward fit of rectangular sachets into a cylindrical vessel. Figure 3 is a sketch of a cooling vessel designed to accommodate four 4 in. × 4 in. (50 ml.) sachets in the apparatus described by Pegg and Trotman (1959). Of the alternatives to these sachets, glass ampoules remain the best for small volumes, though polyethylene ampoules should be satisfactory for short-term storage.

As a biological test, bone marrow was obtained from mice (C- female), frozen with 12·5% dimethyl sulphoxide in 5 ml. polyethylene ampoules or 20 ml. sachets, and kept at −79°C. for a week. It was then thawed and injected intravenously into lethally irradiated isologous mice (700 r; LD_{99} at 30 days). All mice received the same cell dose (100,000 in 0·5 ml.). The 30-day survival with sachet-frozen cells was rather better (40/56) than those frozen in ampoules (44/84), indicating that although the volume of the sachets was four times larger, at least as many, and perhaps more, haemopoietic stem-cells were preserved.

[1]We used a Zeva heat-sealer, supplied by S. Kempner Ltd., London.

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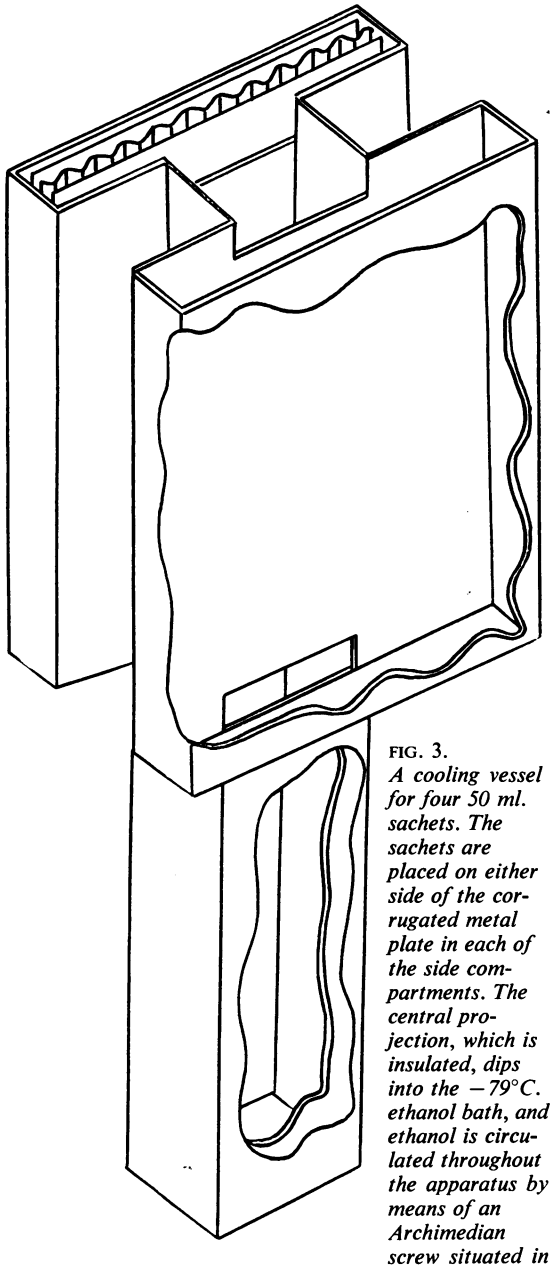

FIG. 3.
A cooling vessel for four 50 ml. sachets. The sachets are placed on either side of the corrugated metal plate in each of the side compartments. The central projection, which is insulated, dips into the −79°C. ethanol bath, and ethanol is circulated throughout the apparatus by means of an Archimedian screw situated in the central tube.

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Macroscopic demonstration of infarction in fresh brain slices

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The changes in the naked-eye appearance of gross sections of unfixed brain following cerebral infarction are often subtle. Although they rarely confuse the experienced observer, it is useful to be able to confirm their presence and demonstrate the extent of the infarcted areas at the time of necropsy. Associated with the many changes occurring in injured tissue, there is a striking fall in the hydrogen ion concentration, and this has been utilized to provide a visual demonstration of the infarcts.

PROCEDURE

The cut slices of brain are laid out in a dish and sufficient B.D.H. Universal indicator added to form a thin film over the surface. The plastic dropping bottles, in which 100 ml. of solution is supplied, are most convenient for this purpose. The cut surface of the brain is irregular, and the indicator tends to pool, but this does not interfere with the interpretation of any changes that may occur. Because the indicator is in aqueous solution and does not easily penetrate the brain tissue, two minutes should be allowed before any colour change is looked for. The colour of the indicator when added is deep green. On normal brain tissue it is a brighter yellow green indicative of a *p*H of 7. Areas of infarction show in sharp contrast as bright red, the *p*H of the tissue in these areas being less than 4. The poor penetration of the dye into the brain tissue has the advantage that the indicator can be rinsed off, and blocks taken for histological examination without being affected by this prior treatment.

LIMITATIONS

There must be a minimum time between the occurrence of a vascular occlusion and the death of the patient for the *p*H change to occur. The duration of this period is not known, but it is believed to be short and probably of the order of an hour or two. On the other hand there will be a maximum time between the death of the patient and examination of the brain after which non-infarcted tissue will have degenerated to such an extent as to be indistinguishable by this method from areas of infarction. From the cases studied so far it can be stated that infarcts can still be demonstrated 36 hours after death but in a necropsy four days after death the whole of the brain tissue turned the indicator red.

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